# FIG. 1

*glyQS* leader

Stem I

Specifier Loop

Terminator

Anti-terminator

T Box

T Box

Stem II

Stem III

IIA/B Pseudoknot

{A U U U U A A A A A}

# FIG. 2



tyrS LEADER
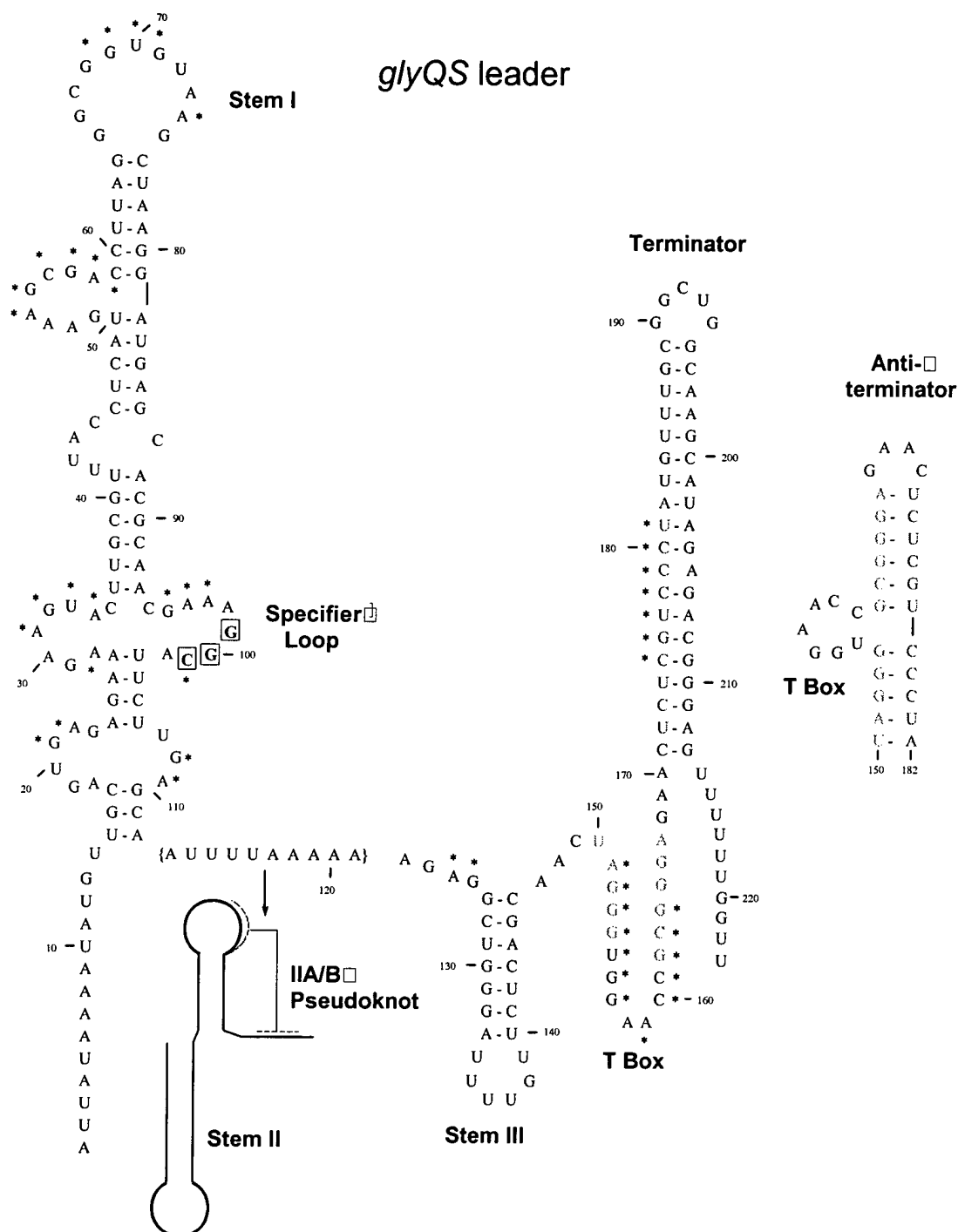
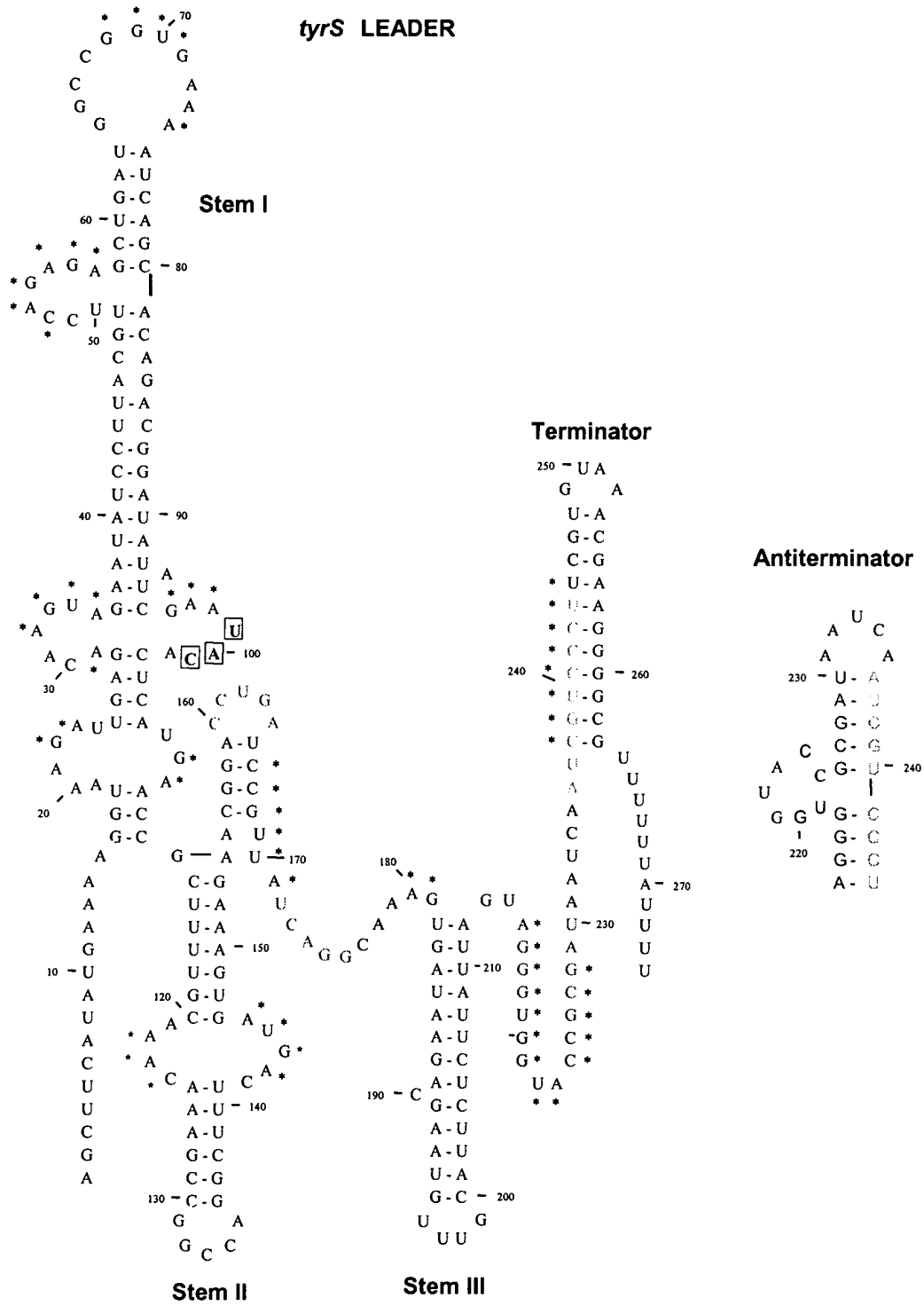Stem I

Terminator

Antiterminator

Stem II

Stem III

# FIG. 3: Panel A

```
B.sub tyrS  AGCTTCATATGAAAAGGTAAAGATTGAGA--CAAGTAGA-ATATCCT-------TACGT-----TCCAGAGA--GCT---GAT
B.ant glyS  ATTATTAATATAAGTAGCGATGACGGACT-TATAAGTACTTGC----------ACA------AAAAGCGA--TTC---AGG
B.cer glyS  ATTATTAATATAAGTAGCGATGACGGACT-TATAAGTACTTGC----------ACA------AAAAGCGA--TTC---AGG
B.hal glyS  AATGTTATATTTCAATGCTATGACGGACGA--ACAGTACTTGATTCCT------TTTACAT----AAAAGCGA--ACC---TAG
B.ste glyS  AAATCATATGGATCGCGATGGATC--AATAGTAGTTAACCCTC----------TCTTC----CCAAGCGA--GCC---GGG
B.sub glyS  TATTATTAAATATGTTGCAGTGAGACAAAA--GAAGTACTTGCGTTTA------CCTCAT----GAAAGCGA--CCT---TAG
C.ace glyS  ATAATTAATATCTATACAATGACAAAGAT--AGAAATTGTATTTT-------CTT-----CAAAGAGA--GGC---TGT
C.hyd glyS  AATTAATAATGGTCAGTGAACCGGTA--GGAGTACTGTGATTT---------CCCT-----TAAAGAGA--GCC---GGG
D.rad glyS  -------GGAGGCGTTGAACCGCA--GGAGTACCG-CGAAGA---------GCCCCCA---ACGAGCGA--GCC---TGA
E.fae glyS  GAGAAGTAAATACGTACGAAGAAAAAGA--GAAGTAAAAAGAACCC-------TCTGTT----AAGCGAA-TCT---GG
L.lac glyS  GCCTTGACAAATGGGAAAATAGAGCTAG---AATTTATA-A------------GGT------AGCGA----CTC---GAA
L.mon glyS  ATATCACTAATAGGTACGTTGAAGGAAA--ATAGTAACAAAAAGCT-------CT-AT----TTTTAGCGA--GTC---CGG
S.aur glyS  AGTGCACAAACACATTAATTTTACTTGCC-TTTAAATAATCTATCAA------TT--*GTACAGCGA--GTT---AAG
S.equ glyS  TTTGTGATAAACTAACCAATAG-GAAAGA--AAATAGCAGG--------TTTCTGATC---TAGACGGA--GTT---CGG
S.mut glyS  ---------GTTAAGAAAGA----------GACT--TTTGT---------GGCGTTTC---TGCAGCGA--ACC---TGA
S.pne glyS  ATTTTTGATATATAAGTCAGCAGGAAAGA-------AAGTCTT--AT-----GGCGTTCT--TCAAGCGA--GCT---TGG
S.pyo glyS  GCTTTATGCTAAACTAGACTCTAGCGAAAA-------AGGATGCAAG------TATCTTATC--TAAAGCGA--GTT---CGG
```

# FIG. 3: Panel B

```
B.sub tyrS  ---GGCCGGTGAAA--ATC---AGCACAGA------CGGATATATC--GAA--TACA---CTCATGAACC-----------
B.ant glyS  ---GATAGTGAAAG-CCT---GAAGCC--------------------GCAAG--GAA-ACGGCA--GTCTCGAGC------------
B.cer glyS  ---GATAGTGAAAG-CCT---GAAGCC--------------------GCAAG--GAA-ACGGCA--GTCTCGAGC------------
B.hal glyS  ---GATGGTGAGAG-CTA---GGGATGTAAA----------------GCAAG--GAA--GGCA--GTCTTGAGCA-TGAA-----
B.ste glyS  ---GACGGTGGAAG-CCC---GGCGAAGA------------------CATCAAG--GAA--GGCA--GTCGGAGCG----------
B.sub glyS  ---GGCGGTGTAAG-CTA---AGGATGAG------------------TGGTTAAT--GAA-ACGGCA--TTCTTGAGCAA-----
C.ace glyS  ---GGAGGGTGTAA--ACG---GTCAAG-------------------CACGCAAC--GAT--AGGCA--TTCTTCGAGTA------
C.hyd glyS  ---GGCTGGTGTGA--ACC---GGTAGGG------------------AAAATTCAGT--AGT--GGAG-TCTTCGAGTA------
D.rad glyS  ---GACGGTGAGAG-TCA---GGCAGGGTGA----------------A-TAAACGGT--GAA--GGCG--CCKGGAGCCG-----
E.fae glyS  ---GAGAGTGGGAG-CC----AGAAACA-------------------GGCGCGACGG--GAA--AGGCA--GGKGGAGCC-----
L.lac glyS  ---GATAGTGAAAG-TTC---GAGAAC--------------------CGGAACTTTT--GAA--AGGCA--CTTGGAGTACG----
L.mon glyS  ---GTTGGTGTGAG-CCG---GATATTTA------------------AATAA--T----GGCT--TAACTT-------------
S.aur glyS  ---GATAGTGTAAG-CTT---AACAA---------------------ACTTTTTGT--GAA--GGCG--TTCTGGAGTA------
S.equ glyS  ---GGCTGGTGAGAG-CCG---AGTGAT-GG--T-------------T---AAGATT---GGC--GCAACGAATC---------
S.mut glyS  ---GAGAGTGTAAG-TCA---GGTGAAACAAA---------------ACTGCTGGTT--GGCG--TTCTCTA-----------
S.pne glyS  ---GATAGTGGGAG-CCA---ACTAGGGCAAA---------------ATAAAGGACT---GGCA--CTTTCT------------
S.pyo glyS  ---GGTAGTTAGAG-CCG---AATGGTAGGA----------------CTGCAGATT----GGCG--CTTACGTTT--------
```

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{DHD1025.DOC;2}

## FIG. 3: Panel C

```
B.sub tyrS  GCT-------TTTGC--AAAC--AAAGCC-----GGCCA-------GGCTTT-----CAGTA---GTGAA-------AGA
B.ant glyS  ------------------------------AATACGTGATAA--------------------------------
B.cer glyS  ------------------------------AATACGTGATAA--------------------------------
B.hal glyS  ------------------------------CGATGAAAGAA----------------------------------
B.ste glyS  ------------------------------GAAATGGCAAAA--------------------------------
B.sub glyS  -------------------------------------------------------------------------
C.ace glyS  TTT----------TT---------------AAAA--------------GA----------AAA
C.hyd glyS  GCA------GGAGGAAA--CCC-----------CAAG------GGG------AGTAAAGCC------TGC
D.rad glyS  -------------------------------------------------------------------------
E.fae glyS  -------------------------------------------------------------------------
L.lac glyS  ----AAAACTGTAATGAAC-----------ACAAAT-------AAAGTAAAAA-
L.mon glyS  ------------------------------CAGCGAAATCAA--------------------------------
S.aur glyS  ------------------------------ATTTTAAAATAAA------------------------------
S.equ glyS  ------------------------------AAGAGTAGGCTCA------------------------------
S.mut glyS  CTT---------GGCT--------------AAT----------AGCC-------AAG
S.pne glyS  -------------------------------------------------------------------------
S.pyo glyS  GGG--CAGTGT-GATTAAG---------------TATA-----TTT-GTCAATATTG--CCC
```

## FIG. 3: Panel D

```
B.sub tyrS  -ACGGA--------CCTGA-----TCCGTTATCAGG----------
B.ant glyS  ---------------------------------------------
B.cer glyS  ---------------------------------------------
B.hal glyS  ---------------------------------------------
B.ste glyS  ---------------------------------------------
B.sub glyS  ---------------------------------------------
C.ace glyS  -GC--------AGG--------GCTATTGCC--------------
C.hyd glyS  ---------------------------------------------
D.rad glyS  ---------------------------------------------
E.fae glyS  ---------------------------------------------
L.lac glyS  ---------------------------------------------
L.mon glyS  ---------------------------------------------
S.aur glyS  ---------------------------------------------
S.equ glyS  ---------------------------------------------
S.mut glyS  ---------------------------------------------
S.pne glyS  ---------------------------------------------
S.pyo glyS  ---------------------------------------------
```

## FIG. 3: Panel E

```
B.sub tyrS  --------TAAGACGAATGTTTG-------- --------CATTC-TCTTA------ ----TTA
B.ant glyS  --------AAAGTGA---------------- --------AGTGGAT---------- --------GCACCTTTT------ --------GTGT---- ----ATC
B.cer glyS  --------AGTGGAT---------------- --------GCACCTTTT-------- --------GTGT----------- ----ATC
B.hal glyS  --------AGTGGCC---------------- --------TATGGTGTC-------- --------ATCATA--------- ----GGC
B.ste glyS  --------AGGGGAT---------------- --------GCGTGATT--------- --------TGCGC--------- ----ATC
B.sub glyS  ----TTTTAAAAAGAGGCT------------ --------GGGATTTTGT------- --------TCTC---------- ----AGC
C.ace glyS  --------AGAGTTTTGAGGTGGGC------ --------CTTTTTT---------- --------G------------- ----GCC
C.hyd glyS  ACAACCGGTCGAAAGGTGCTG---------- --------GCGAGG----------- ----CAG
D.rad glyS  --------ACAAACGAAGCT----------- --------GC-------------- ----CAG
E.fae glyS  --------GCCGATGAACA------------ --------CATCGG---------- ----AAG
L.lac glyS
L.mon glyS  --------GGTGGGA---------------- --------ATTGTTT--------- --------TAAT---------- ----TCC
S.aur glyS  --------AGCGAGT---------------- --------GACTA----------- --------C------------ ----ACT
S.equ glyS  --------GGTGTTT---------------- GTAGCTTGCTTGACATCTGTT--- --------TATCAA-CAAG-ATC---- ----AAA
S.mut glyS  --------CTAACA----------------- --------ATCAGATAAA------ --------TGA---------- ----AGT
S.pne glyS  --------GTAGTAT---------------- --------T-TTCAAAAACAA--- --------TGAAGTA------ ----ATA
S.pyo glyS  --------AAAAGATAC-------------- --------TATATAAA-------- --------TGAA--------- ----GTA
```

## FIG. 3: Panel F

```
B.sub tyrS  --------GTAGGGTGGTACCGCGAT----------- --------AATCA---------- --------ATCGT-------- --------CCCT
B.ant glyS  --------AACTAGGGTGGAACCGCGGGC-------- --------AAAC----------- --------GCTCGT------- --------CCCTAG
B.cer glyS  --------AACTAGGGTGGAACCGCGGGC-------- --------AAAC----------- --------GCTCGT------- --------CCCTAG
B.hal glyS  --------AAATAGGGTGGAACCGCGGG--------- --------TTAACT--------- --------CTCGT-------- --------CCCTAT
B.ste glyS  --------AACTAGGGTGGAACCGCGGGAGC------ --------TAC------------ --------GCTCTCGT----- --------CCCTAG
B.sub glyS  --------AACTAGGGTGGAACCGCGGGA-------- --------GAAC----------- --------TCTCGT------- --------CCCTA
C.ace glyS  --------AATAAGGGTGGAACCGCGGAA-------- --------GTAA----------- --------TTTCGT------- --------CCCTT
C.hyd glyS  --------AACCAGGGTGGAACCGCGGAAGG------ --------ATGCC---------- --------CCTTTCGT----- --------CCCTGG
D.rad glyS  --------AACTGGGGTGGAACCGCGCATG------- --------TCC------------ --------CGTGCGT------ --------CCCCGG
E.fae glyS  --------TAGGGTGGAACCGCGA------------- --------TAATTAT-------- --------TCGT-------- --------CCCTA
L.lac glyS  --------ATAAAGGTGGAACCGTGC----------- --------ATTT----------- --------GCAC-------- --------CCTTTGT
L.mon glyS  --------AAATAGGGTGGAACCGCGAG--------- --------CTAACT--------- --------CTCGT------- --------CCCTAT
S.aur glyS  --------AATTTGGGTGGAACCGCGGGTT*------ --------AACTCGT----- --------CCCA
S.equ glyS  TGAAGTAATAAATAAATTAGGGTGGAACCGCGCGT-- --------TTTG----------- --------ACGC-------- --------CCCTA
S.mut glyS  --------AATAAATTAGGGTGGAACCGCGTT----- --------TCA------------ --------AACGC------- --------CCCTA
S.pne glyS  --------AATTAGGGTGGAACCGCGTT--------- --------TCT------------ --------GACGC------- --------CCCTAG
S.pyo glyS  --------ATAAAATTAGGGTGGAACCGCGT------ --------TTTG----------- --------ACGC-------- --------CCCTAG
```

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# FIG. 4

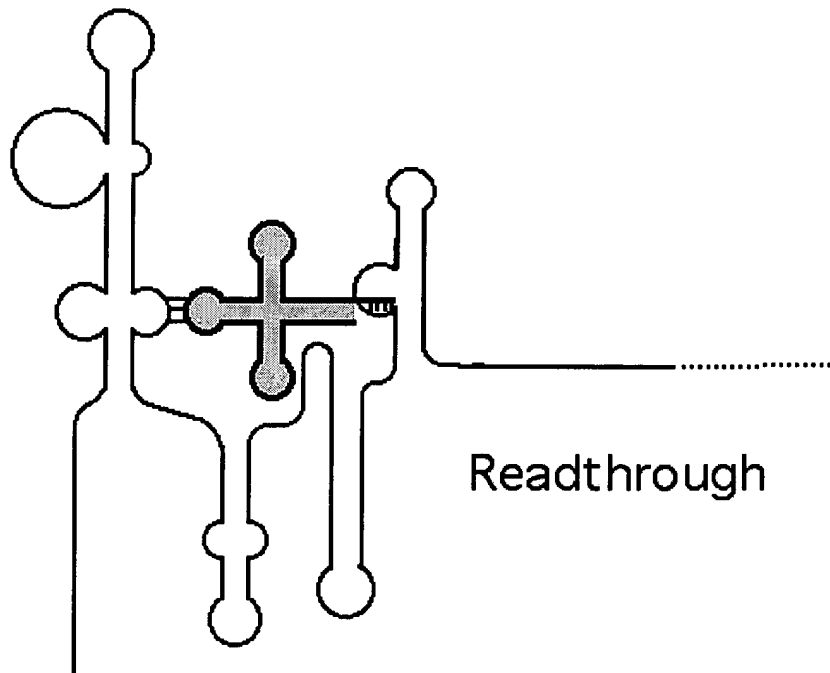| ORGANISMS WITH LEADER SEQUENCES HAVING THE T BOX PATTERN | NCBI GENOME SEQUENCE FILE NAME |
|---|---|
| *Bacillus anthracis* | NC_003997 |
| *Bacillus cereus* | NC_004722 |
| *Bacillus halodurans* | NC_002570 |
| *Bacillus subtilis* | NC_000964 |
| *Carboxydothermus hydrogenoformans* | NC_002972 |
| *Clostridium acetobutylicum* | NC_003030 |
| *Clostridium botulinum* | NC_003223 |
| *Clostridium difficile* | NC_002933 |
| *Clostridium perfringens* | NC_003366 |
| *Clostridium tetani* | NC_004557 |
| *Clostridium thermocellum* | NZ_AABG00000000 |
| *Corynebacterium glutamicum* | NC_003450 |
| *Corynebacterium diptheriae* | NC_002935 |
| *Deinococcus radiodurans* | NC_001263 |
| *Desulfitobacterium hafniense* | NZ_AAAW00000000 |
| *Enterococcus faecalis* | NC_004668 |
| *Enterococcus faecium* | NZ_AAAK00000000 |
| *Geobacillus stearothermophilus* | NC_002926 |
| *Geobacter sulfurreducens* | NC_002939 |
| *Lactococcus lactis* | NC_002662 |
| *Leuconostoc mesenteroides* | NZ_AABH00000000 |
| *Listeria innocua* | NC_003212 |
| *Listeria monocytogenes* | NC_003210 |
| *Mycobacterium leprae* | NC_002677 |
| *Mycobacterium tuberculosis* | NC_000962 |
| *Oceanobacillus iheyensis* | NC_004193 |
| *Oenococcus oeni* | NZ_AAAAZ00000000 |
| *Ruminococcus albus* | NC_003373 |
| *Spiroplasma kunkelii* | NC_003999 |
| *Staphylococcus aureus* | NC_002758 |
| *Staphylococcus epidermidis* | NC_004461 |
| *Streptococcus equi* | NC_002955 |
| *Streptococcus gordoniae* | NC_002979 |
| *Streptococcus mutans* | NC_004350 |
| *Streptococcus pneumoniae* | NC_003098 |
| *Streptococcus pyogenes* | NC_002737 |
| *Streptomyces coelicolor* | NC_003888 |
| *Thermoanaerobacter tengcongensis* | NC_003869 |
| *Thermobifida fusca* | NZ_AAAQ00000000 |

FIG. 5

High tRNA charging

TYR
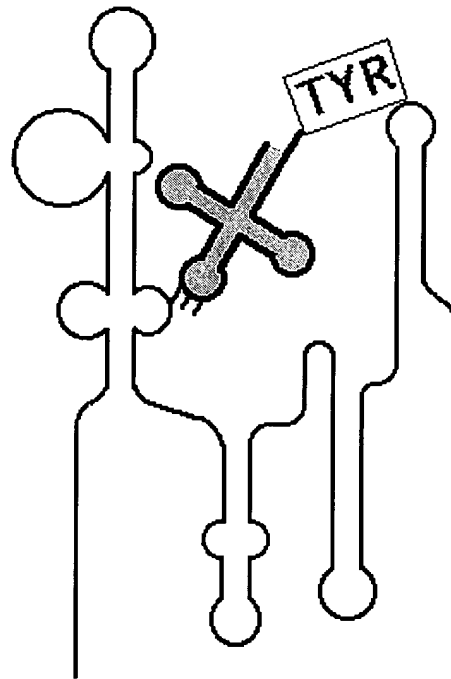
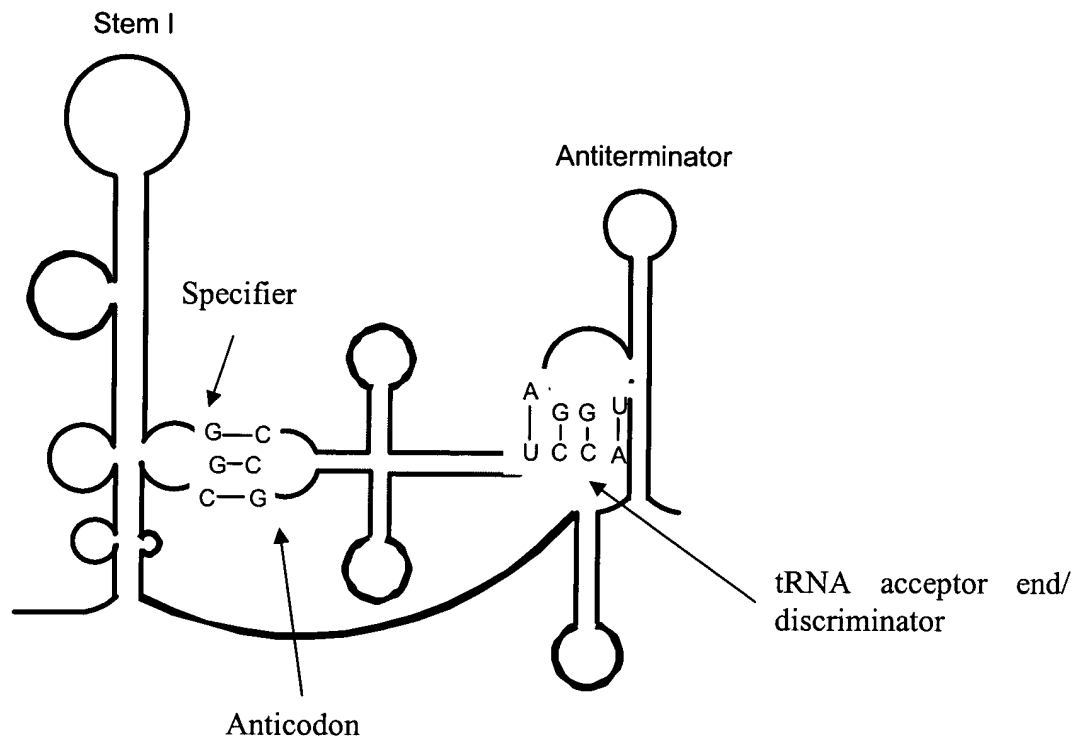Terminate

Low tRNA charging

Readthrough

# FIG. 6

# FIG. 7

5



RNAP

RNAP continues

factor?

codon-anticodon
pairing

antiterminator-
acceptor end
pairing

# FIG. 8

TTGACATTTGGTCCATCTTTTTATATGATCATTTATTATAAAATATGTTGC

-35              -10      +1              +17

T
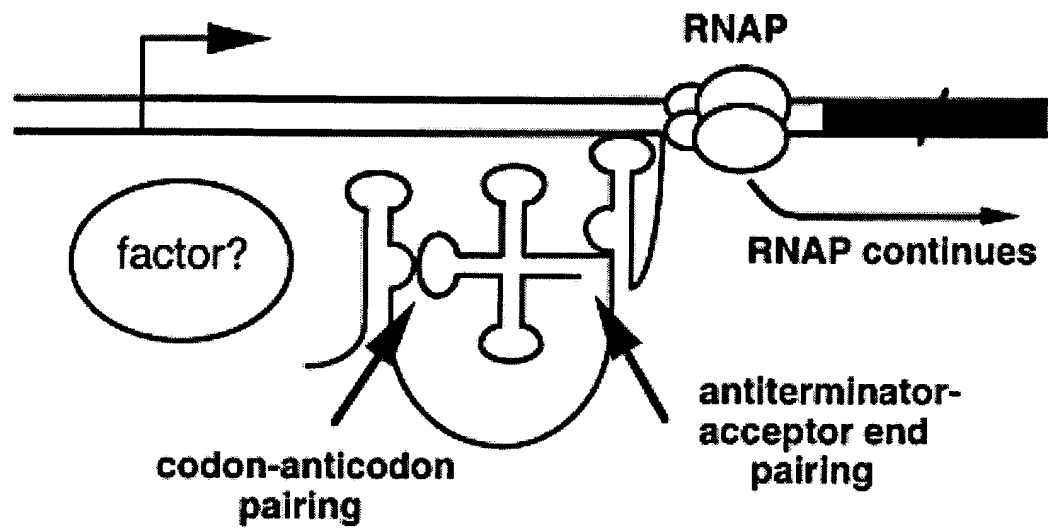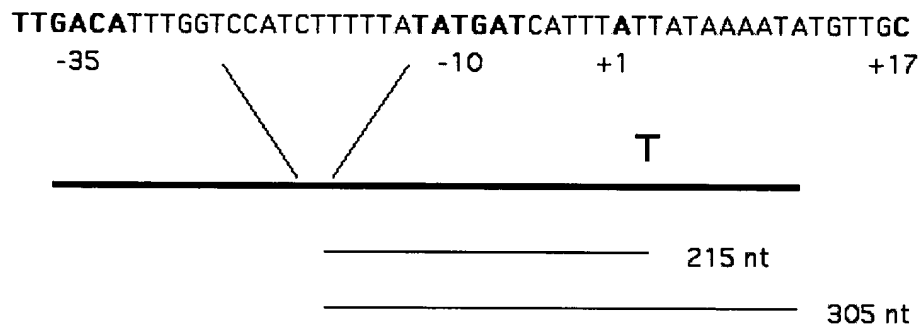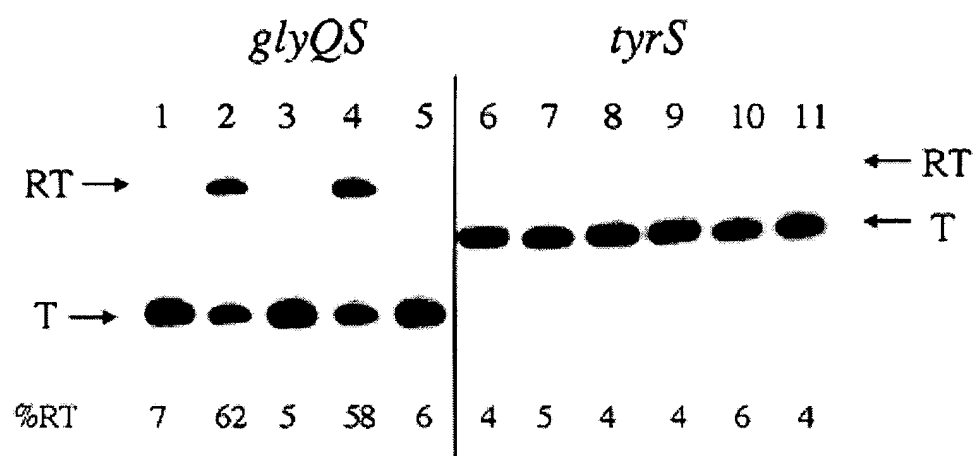
————————————————— 215 nt

———————————————————— 305 nt

# FIG. 9

# FIG. 10
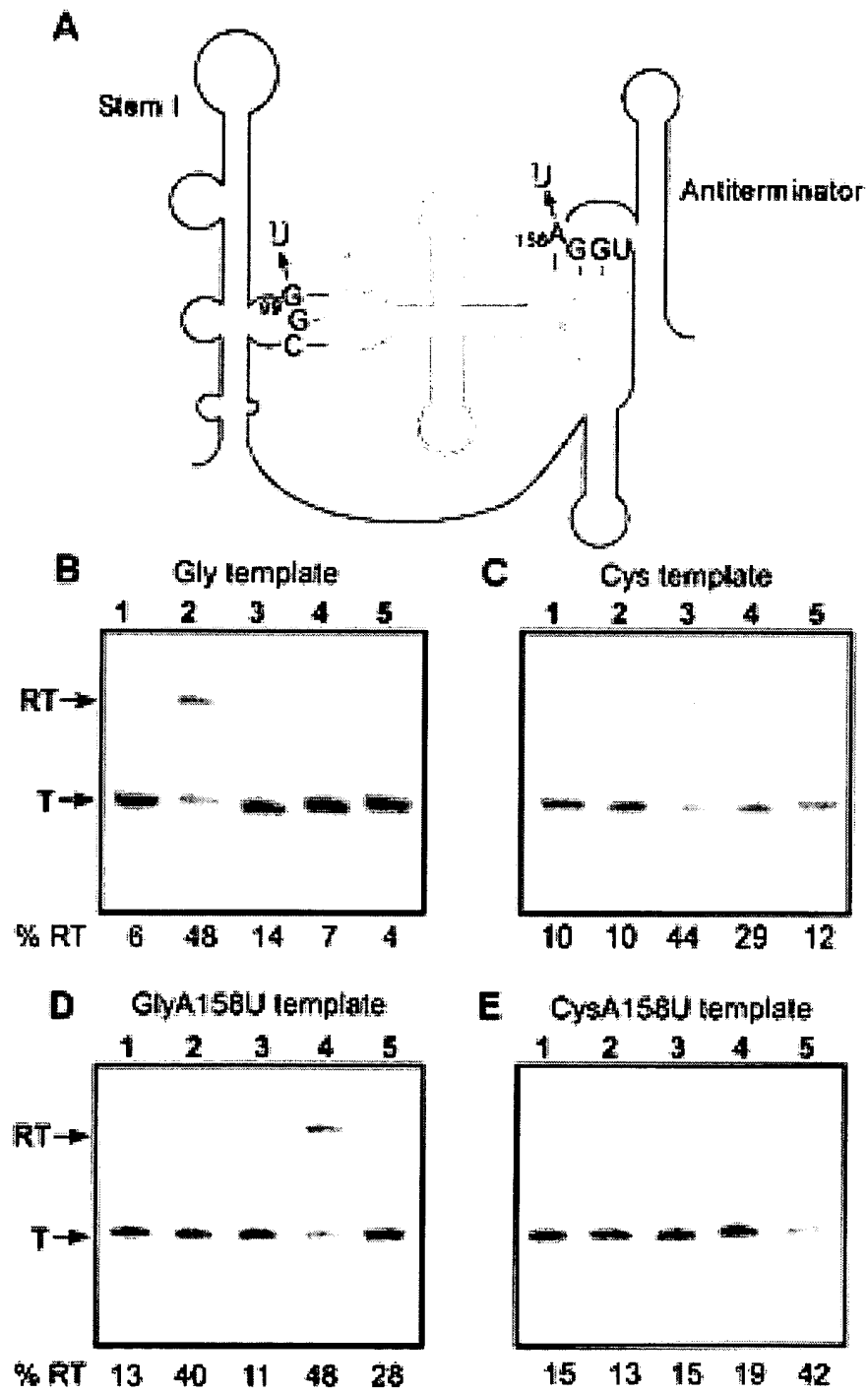
# FIG. 11

Polynucleotide sequence for the *glyQS* gene from *Bacillus subtilis* corresponding to the *in vitro* transcription template: from *B. subtilis* 168.

ATTGATTTATATTACGAAGAATATTCGGGATTGTATTTAAAATCAAAGCGCTTTTTAGATCAAATGGAAAGCATGAA
ACATCTTATGGGTGAAAACAAAAGTTGACATTTGGTCCATCTTTTTATATGATCATTTATTATTAAATATGTTGCAG
TGAGAGAAAGAAGTACTTGCGTTTACCTCATGAAAGCGACCTTAGGGCGGTGTAAGCTAAGGATGAGCACGCAACGA
AAGGCATTCTTGAGCAATTTTAAAAAAGAGGCTGGGATTTTGTTCTCAGCAACTAGGGTGGAACCGCGGGAGAACTC
TCGTCCCTATGTTTGCGGCTGGCAAGCATAGAGACGGGAGTTTTTTGGTTGCTGCCGCAGTCAACTTATGAAAGAAA
AGTGGAGGTGCTTGAAATGAATATTCAAGACATGATTCTAACCTTGCAAAGC

# FIG. 12

Sequence of same region from BR151MA (T to A at +6 position relative to transcription start-site)

ATTGATTTATATTACGAAGAATATTCGGGATTGTATTTAAAATCAAAGCGCTTTTTAGATCAAATGGAAAGCATGAA
ACATCTTATGGGTGAAAACAAAAGTTGACATTTGGTCCATCTTTTTATATGATCATTTATTATAAAATATGTTGCAG
TGAGAGAAAGAAGTACTTGCGTTTACCTCATGAAAGCGACCTTAGGGCGGTGTAAGCTAAGGATGAGCACGCAACGA
AAGGCATTCTTGAGCAATTTTAAAAAAGAGGCTGGGATTTTGTTCTCAGCAACTAGGGTGGAACCGCGGGAGAACTC
TCGTCCCTATGTTTGCGGCTGGCAAGCATAGAGACGGGAGTTTTTTGGTTGCTGCCGCAGTCAACTTATGAAAGAAA
AGTGGAGGTGCTTGAAATGAATATTCAAGACATGATTCTAACCTTGCAAAGC

# FIG. 13

tRNA$^{Gly}$ DNA sequence: (from SubtiList, confirmed by sequencing of region in BR151MA)

GCGGAAGTAGTTCAGTGGTAGAACACCACCTTGCCAAGGTGGGGGTCGCGGGTTCGAATCCCGTCTTCCGCTCCA

# FIG. 14

PCR primers used for preparing *glyQS* template:


GlyQUS1Xba:  ATTGATCTAGATTACGAAGAATATTCGGGATTGTA (contains two
changes from sequence shown in Fig. 12 to introduce an XbaI site (TCTAGA)
at the 5' end of the fragment for generation of the *glyQS-lacZ* fusion
construct)

GlyQDS2H3Pac:  GGGTATTTAATTAAGCTTTTGCAAGGTTAGAATCA (introduces extra
14 nt downstream of *glyQS* sequence shown in Fig. 12 to provide a HindIIIsite
(AAGCTT) for generation of the *glyQS-lacZ* fusion construct)


The PCR primers used to generate the *tyrS* template:

TyrUSBM:  GGCTGGGGATCCGTCAACAATGGAGG
TyrN2b:  CCGCGGAAGGATAAAGCTTCAAGTAAG

# FIG. 15

Polynucleotide sequence for the *tyrS* template DNA (identical for 168 or BR151MA).

```
GGCTGGAGATCTGTCAACAATGGAGGATTAAAAGGCGGCGTTGACACAGGATTTTATTTATGTTAAAAATGATATAG
CTTCATATGAAAAGGTAAAGATTGAGACAAGTAGAATATCCTTACGTTCCAGAGAGCTGATGGCCGGTGAAAATCAG
CACAGACGGATATATCGAATACACTCATGAACCGCTTTTGCAAACAAAGCCGGCCAGGCTTTCAGTAGTGAAAGAAC
GGACCTGATCCGTTATCAGGCAAAGTGATAAGACGAATGTTTGCATTCTCTTATTAGTAGGGTGGTACCGCGATAAT
CAATCGTCCCTTCGTGTAAACGAAGGGGCGTTTTTTATTTTAATTAAAAAAGGAGCTTTATCTTATGACTAACTTAC
TTGAAGACTTATCCTTCCGCGG
```

# FIG. 16

The oligos used to generate tRNA<sup>Tyr</sup>:

```
5':  TyrUSBM:  GGCTGGGGATCCGTCAACAATGGAGG
3':  TyrN2b:   CCGCGGAAGGATAAAGCTTCAAGTAAG


5':  tRNATyrUST7:  TAATACGACTCACTATAGGAGGGGTAGCG (the tRNA sequence
starts with GGAGGGGTAGCG)
3':  tRNATyrDS:  TGGTGGAGGGGGGCAGATTCG
```

The oligos used to generate tRNA<sup>Gly</sup>:

```
5':  GlytRNAUS:  TAATACGACTCACTATAGCGGAAGTAGTTCAGTGG
(the first part of this oligo is the T7 promoter; the tRNA starts
with GCGGAAGTAGTTCAGTGG)
3':  GlytRNADS:  TGGAGCGGAAGACGGGATTCGAAC
```

# FIG. 17

tRNA<sup>Tyr</sup> DNA sequence.

GGAGGGGTAGCGAAGTGGCTAAACGCGGCGGACTGTAAATCCGCTCCCTCAGGGTTCGGCAGTTCGAATCTGCCCCC
CTCCACCA